

In Vitro Activities of Linezolid against Clinical Isolates of *Mycobacterium tuberculosis* That Are Susceptible or Resistant to First-Line Antituberculous Drugs

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We evaluated 117 isolates of *Mycobacterium tuberculosis* for susceptibility to linezolid by the proportion and E-test methods. Linezolid showed high in vitro activity, with all the strains inhibited by ≤ 1 μ g of the drug per ml. E-test MICs were at least 4 dilutions lower than their equivalents by the standard proportion method.

Linezolid is an oxazolidinone whose mechanism of action involves inhibition of protein synthesis at a very early stage (7). This new drug has shown good activity against some gram-positive bacteria, including resistant staphylococci, enterococci, and pneumococci (2, 5, 9). However, information in the literature regarding the activity of linezolid against *Mycobacterium tuberculosis* strains is scarce.

We evaluated the in vitro activities of linezolid using the standard and E-test methods against 117 clinical isolates of *M. tuberculosis* with different levels of susceptibility to first-line antituberculous drugs.

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The active substance as reference powder and the linezolid E-test strips were provided by Pharmacia Upjohn (Barcelona, Spain). Stock solution was prepared at 10,000 μ g/ml in distilled water. Aliquots of the oxazolidinone were frozen at -70°C until use. *Staphylococcus aureus* strain ATCC 29213 was used as a quality control to assure the potency of the drug and the E-test activity.

A total of 117 isolates of *M. tuberculosis* from different patients were selected from our laboratory collection (isolates collected in 1988 to 2000). Susceptibility testing on first-line antituberculous drugs (streptomycin, isoniazid, rifampin, and ethambutol [SIRE]) was performed by the agar proportion method at a reference laboratory. Of all the strains tested, 44 strains (38%) were resistant to at least one SIRE (R-SIRE), 12 strains were resistant to both isoniazid and rifampin, while the remainder were susceptible to the four SIRE drugs (S-SIRE).

To prevent bias in the performance of the study, there was a single initial inoculum for each isolate for both the proportion and E-test methods. This inoculum was prepared in Middlebrook 7H9 and was adjusted to be equivalent to that of a McFarland no. 3 standard. Furthermore, both methods were performed in a double-blind manner.

The agar proportion method was performed as recommended by the National Committee for Clinical Laboratory Standards (6). Briefly, linezolid was added to 7H10 agar medium (Difco) supplemented with OADC (oleic acid, albumin, dextrose, and catalase) (Becton Dickinson) at 50 to 56°C by doubling the dilutions to yield a final concentration of 0.125 to 4 μ g/ml. Five milliliters of each concentration of antimycobacterial agent-containing medium was dispensed into plastic quadrant petri dishes. As a growth control, one quadrant in each plate was filled with 7H10 agar medium with no drug. The inoculum of each isolate was prepared by diluting the initial inoculum in Middlebrook 7H9 until the absorbance was reduced to that of an equivalent of McFarland no. 1 standard. Final suspensions were performed by adding Middlebrook 7H9 and preparing 10^{-2} and 10^{-4} dilutions of the standardized suspensions. Upon solidification of the medium, 0.1-ml portions of the dilutions were placed on each quadrant of the agar plates, and the agar plates were sealed in plastic bags and incubated at 37°C for 3 weeks. The MIC of each isolate was the lowest concentration of the antimycobacterial agent that inhibited >99% of the colonies growing on the drug-free control. *M. tuberculosis* ATCC 27294 (H37Rv strain) was used as a control strain.

For testing each isolate by the E-test method, two Middlebrook 7H11 agar plates (100-mm diameter) supplemented with 10% OADC (Difco) were inoculated by swabbing with the initial suspension (McFarland no. 3 standard) of mycobacteria and incubated at 37°C with 5% CO_2 for 24 h. After incubation, one E-test strip was placed on the first plate for each isolate tested. No strip was placed on the second plate, which served as a growth control. The plates were sealed in plastic bags and incubated under the same conditions for 7 to 10 days, after which the MIC was read. The MIC was the value where the growth inhibition ellipse intersected the strip or as specified in the 1997 AB BIODISK E-test technical guide no. 6 for *M. tuberculosis*. For a control, each experiment included susceptibility testing of *M. tuberculosis* ATCC 27294 (H37Rv strain).

The range of MICs, the MIC at which 50% of the isolates are inhibited (MIC_{50}), the MIC_{90} , and the geometric mean of MIC of linezolid obtained by the proportion method are shown in Table 1. All the isolates tested, including isolates that were

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TABLE 1. In vitro activities of linezolid against 117 clinical isolates of *M. tuberculosis*^a

<i>M. tuberculosis</i> isolates (no. of isolates)	MIC (μg/ml)			Geometric mean
	Range	50%	90%	
Susceptible to first-line drugs (73)	0.25–1	0.5	0.5	0.524
Resistant to first-line drugs (44)	≤0.125–1	0.5	1	0.477
Resistant to one first-line drug (25)	≤0.125–1	0.5	1	0.529
Resistant to multiple first-line drugs (19)	0.25–1	0.5	0.5	0.417
All (117)	≤0.125–1	0.5	1	0.506

^a The MIC of strain H37Rv (ATCC 27294) was 0.25 μg/ml.

susceptible to SIRE and isolates that were resistant to SIRE, were inhibited at ≤1 μg/ml and had a geometric mean of 0.506 μg/ml. The level of resistance to SIRE did not increase the linezolid MICs of the strains tested. Although the MIC₉₀ of R-SIRE strains was 1 dilution greater (1 μg/ml) than that of S-SIRE strains (0.5 μg/ml), the geometric mean of resistant isolates was slightly lower (0.477 μg/ml) than susceptible ones (0.524 μg/ml).

The range of MICs, MIC₅₀, and MIC₉₀ using the E-test method were ≤0.016 to 0.023, ≤0.016, and 0.016 μg/ml, respectively. All isolates had an MIC equal to or lower than 0.023 μg/ml, with 88% of the strains showing inhibition ellipses under the lowest MIC of the strips (0.016 μg/ml). Concordance between MICs of the proportion and E-test methods is shown in Table 2. E-test MICs were at least 4 dilutions lower than their equivalents by the proportion method.

Therapy with first-line antituberculous drugs has led to a significant reduction in the morbidity and mortality of tuberculosis. However, the currently available therapeutic regimens have the inherent disadvantages of long-treatment duration, patient noncompliance, and risk of drug resistance (1, 4).

In our study, linezolid showed excellent in vitro activity against all the strains tested (MICs ≤ 1 μg/ml), including those resistant to SIRE. To our knowledge, there is only one other

study of the in vitro activity of linezolid against clinical strains of *M. tuberculosis* (9). However, these researchers tested only five isolates that were susceptible to SIRE and five isolates that were resistant to SIRE, with an MIC₉₀ of 0.5 and 2, respectively, and all the strains had an MIC of 2 μg/ml (concentration of 1 μg/ml was not tested). Although only a few strains were tested, the results were similar to ours.

Our results show that the E-test MICs were at least 4 dilutions lower than those of the standard proportion method. This showed a lack of concordance of the E-test method with the standard procedure.

Despite the high in vitro activity of linezolid against clinical strains of *M. tuberculosis*, there are no clinical trials evaluating its in vivo efficacy. In 1999, Cynamon et al. reported a somewhat lower activity of this drug compared with isoniazid in a murine model (3). Two years later, Valencia et al. described a patient infected with a recurrent multidrug-resistant *Mycobacterium bovis* (species related to *M. tuberculosis*) strain and treated successfully with linezolid and another five drugs during an 11-month period (8). However, the potential toxicities (e.g., myelosuppression) and the high cost (approximately \$100 per day) of linezolid could limit the use of this drug for extended periods of time, as is the case in the treatment of tuberculosis.

In summary, linezolid is very active against *M. tuberculosis*, including those strains that are resistant to conventional antimycobacterial drugs. The potential role of this agent in the treatment of tuberculosis requires further clinical evaluation.

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TABLE 2. Distribution of MICs of linezolid by proportion and E-test^a

Proportion method MIC (μg/ml)	Concordance ^b between proportion method MIC and the following E-test method MIC (μg/ml):		
	≤0.016	0.016	0.023
≤0.125	1 (100)	0	0
0.25	9 (100)	0	0
0.5	89 (95)	3 (3)	2 (2)
1	4 (31)	9 (69)	0

^a The MIC of strain H37Rv (ATCC 27294) was ≤0.016 μg/ml.

^b Each value is the number of isolates in each proportion method MIC range that has the indicated E-test method MIC. The percentage of isolates in each proportion method MIC range is shown in parentheses.